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Quantitative Analysis of Chemically Modified Starches by 1H-NMR Spectroscopy

Graaf, R.A. de; Lammers, G.; Janssen, L.P.B.M.; Beenackers, A.A.C.M.

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Conclusion

Blue values, reducing values, total carbohydrate, average degree of polymerisation and MS varied between fractionated PPA digests because of differing amylose:amylopectin ratios and MS. A most important feature observed in this study was the higher MS in the polysaccharides fraction than in the oligosaccharides. This may indicate greater safety compared to the reversed situation as found in the case of wheat starch [2].

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Addresses of authors: *B. M. N. Modh. Azemi**, School of Industrial Technology, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia, and *M. Wootton,* Department of Food Science and Technology, University of New South Wales, Kensington, Australia 2203.
*Address of correspondence.

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Quantitative Analysis of Chemically Modified Starches by ^{1}H-NMR Spectroscopy

R.A. de Graaf, G. Lammers, L.P.B.M. Janssen, and A.A.C.M. Beenackers, Groningen (The Netherlands)

A quantitative ^{1}H-NMR method for the determination of the Molar Substitution (*MS*) of acetylated and hydroxypropylated starches was developed and tested for *MS* ranging from 0.09 to 0.5. Results were checked using the Johnson method and a titration method for hydroxypropylated and acetylated starch, respectively. Hydroxypropylated starch was produced using both a static mixer reactor (SMX type) and a co-rotating twin screw extruder. Acetylated starch was produced using a counter-rotating twin screw extruder. Quantitative analysis results of the ^{1}H-NMR method were in good agreement with traditional analysis methods for all samples tested. Main advantage of the ^{1}H-NMR method is the considerable time saving as compared to the traditional analysis methods.

Quantitative Analyse von chemisch modifizierten Stärken durch ^{1}H-NMR-Spektroskopie. Eine Bestimmungsmethode des Substitutionsgrades von acetylierter und hydroxypropylierter Stärke mittels ^{1}H-NMR auf molare Substitution (*DS*) von 0,09–0,05 wurde entwickelt. Die hydroxypropylierte Stärke wurde in einem Static-Mixer (SMX) und einem selbstreinigenden Doppelschnecken-Kneter hergestellt. Die acetylierten Stärken wurden in einem gegenläufigen engschließenden Doppelschnecken-Kneter gewonnen. Die hydroxyopropylierten Stärkeprodukte wurden mit der *Johnson*-Methode und durch ^{1}H-NMR analysiert. Die acetylierten Stärkeprodukte wurden durch Titration und ^{1}H-NMR untersucht. Beide Methoden erbrachten ähnliche Ergebnisse mit guter Korrelation.

1 Introduction

Nowadays, large quantities of chemically modified starches are used in industrial processes. Two important product groups are the starch ethers and the starch esters. In this study hydroxypropylated and acetylated starches were used.

Starch is a mixture in variable proportions of the linear poly-(1,4)-α-D-glucan, amylose, and the branched molecule, amylopectin, where linear (1,4)-α-D-glucan chains are connected through (1,6)-α-linkages (*Blanshard*) [1]. The alcohol groups on the anhydroglucose units of both polymers react under alkaline conditions with propylene oxide (*Roberts*) [2] or vi-

nylacetate (*Lammers* et al.) [3]. This way, hydroxypropylated and acetylated starches are produced, respectively (Figure 1). The average number of substituents per D-glucose unit is given by the Molar Substitutions (*MS*). Thus, an *MS* of 1.0 means that on average one substituent is present per D-glucose unit (Figure 1). Since three reactive hydroxyl groups are present in a D-glucose unit, the maximum *MS* is 3.0. In case of hydroxypropyl starch the *MS* can be higher because the hydroxypropyl group can react with another propylene oxide, thus forming poly hydroxypropyl groups.

Various processes for the production of starch derivatives exist (*Ruitenberg* and *Solarek*) [4], each with their own advantages. The samples analysed here resulted from processing of concentrated starch solutions in extruders and static mixer reactors (*Lammers* et al. [5] and *de Graaf* et al. [6]). As part of a larger research program to determine the feasibility of different reactor types for starch derivatization, a direct method for the analysis of modified starches was developed.

2 Experimental

2.1 Materials and Methods.

Hydroxypropyl starch was produced both in a static mixer reactor (*Lammers* et al. [5]) and in a co-rotating twin screw extruder (*de Graaf* et al. [6]). Acetylated starch was produced in a counter rotating closely intermeshing twin screw extruder. The static mixer reactor was fed with an aqueous slurry of native potato starch (AVEBE, food grade), the extruder was fed with native potato starch with a moisture content of 15% by weight. Vinylacetate and propylene oxide were a pro analysis grade from Merck, Germany. Sodium hydroxide solutions were used as a catalyst. Analysis of the starches were carried out using a 200MHz Varian Gemini NMR spectrometer.

2.2 Sample preparation

Techniques used for preparing the samples were partly taken from *Marsman* et al. [7]. Samples from the static mixer reactor

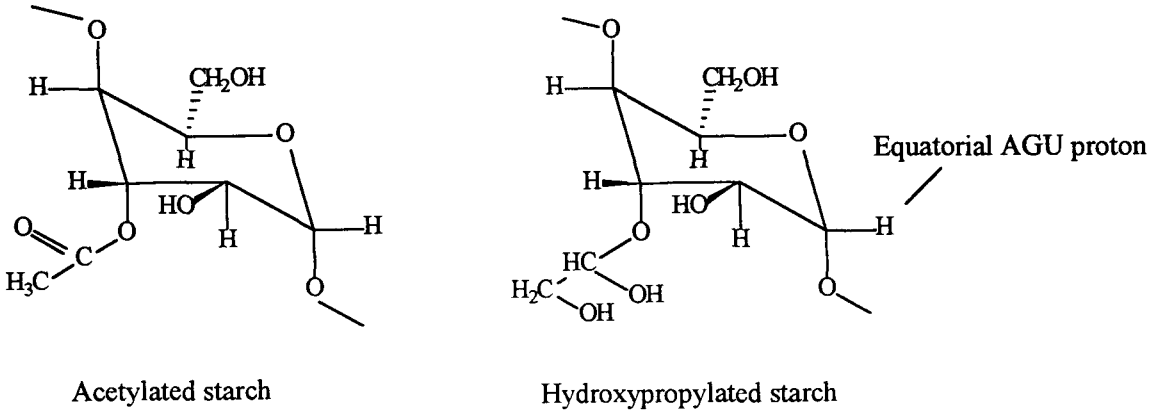
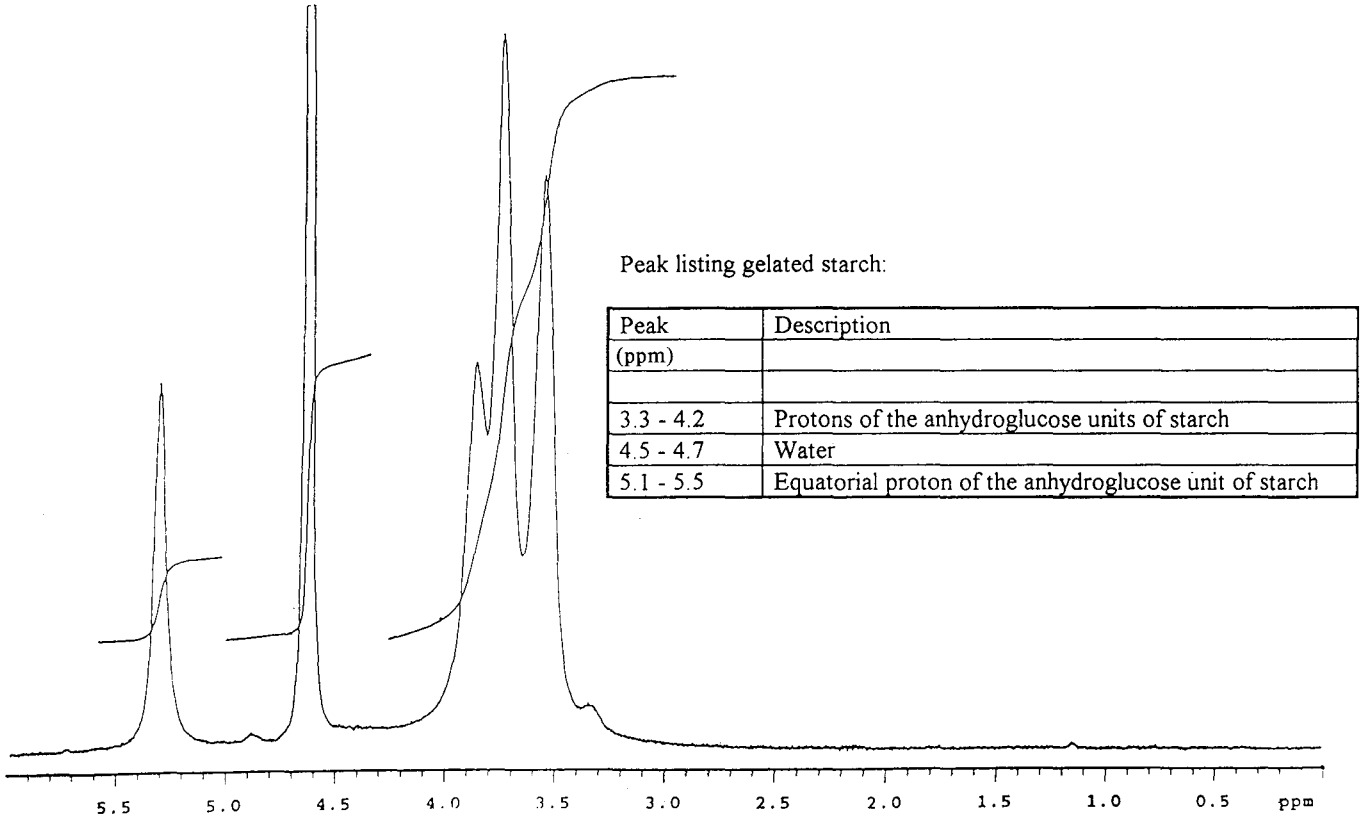


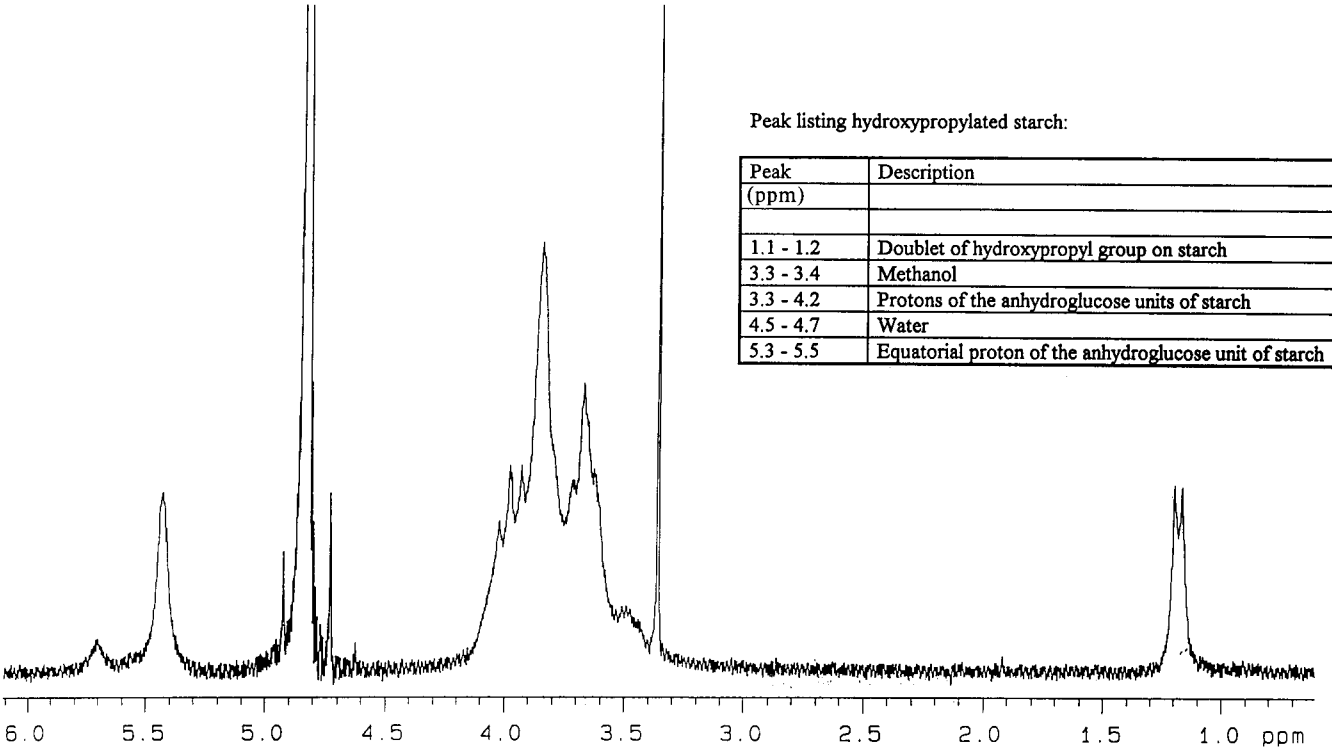
Figure 1. Chemical structures of acetylated and hydroxypropylated starch.



Peak listing gelated starch:

Peak (ppm)	Description
3.3 - 4.2	Protons of the anhydroglucose units of starch
4.5 - 4.7	Water
5.1 - 5.5	Equatorial proton of the anhydroglucose unit of starch

Figure 2. ^{1}H-NMR spectra of gelatinised starch.



Peak listing hydroxypropylated starch:

Peak (ppm)	Description
1.1 - 1.2	Doublet of hydroxypropyl group on starch
3.3 - 3.4	Methanol
3.3 - 4.2	Protons of the anhydroglucose units of starch
4.5 - 4.7	Water
5.3 - 5.5	Equatorial proton of the anhydroglucose unit of starch

Figure 3. ^{1}H-NMR spectra of hydroxypropyl starch.

Peak listing acetylated starch:

Peak (ppm)	Description
2.0 - 2.1	Methyl protons of the acetyl group on starch
3.2	Methanol
3.3 - 4.2	Protons of the anhydroglucose units of starch
4.5 - 4.7	Water
5.1 - 5.5	Equatorial proton of the anhydroglucose unit of starch

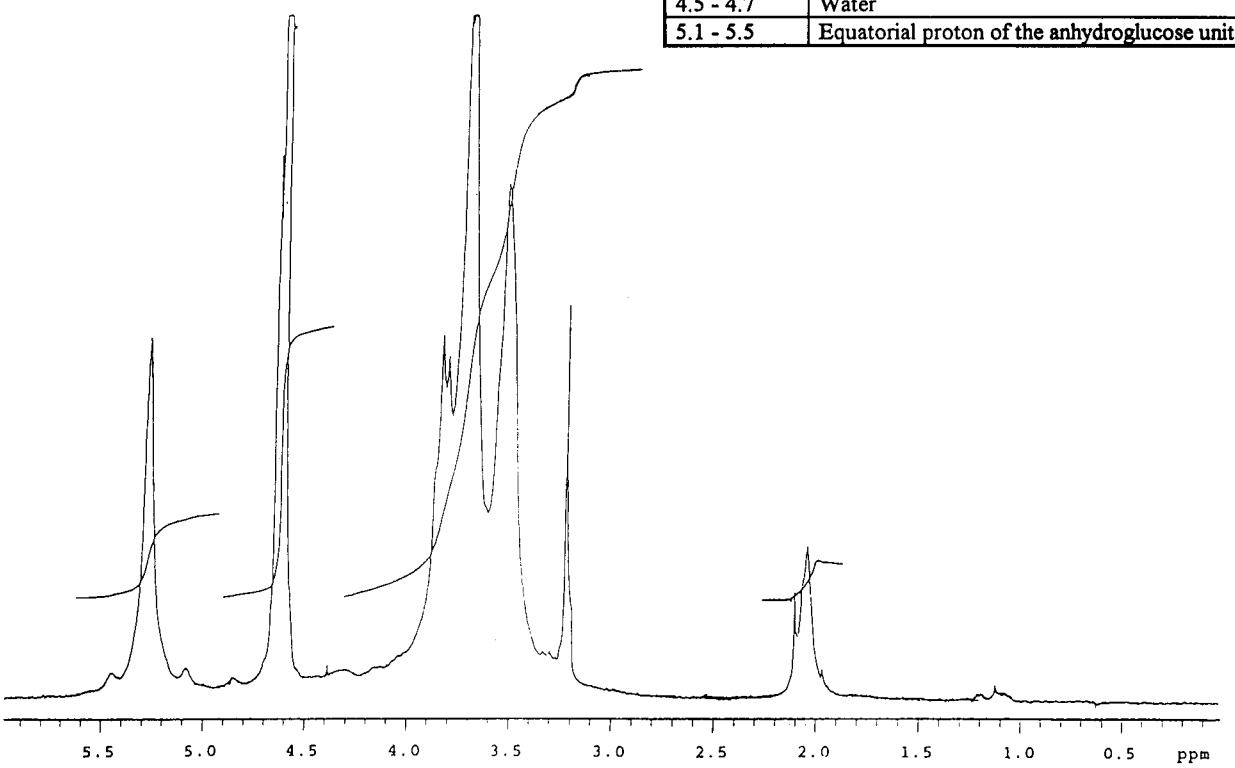


Figure 4. ^{1}H-NMR spectra of acetylated starch.

were quenched in cold water, neutralized with hydrochloric acid and then precipitated in cold (−10°C) acetone. The precipitated starch was washed thrice with cold acetone and then dried in a vacuum stove set at 80°C. Samples from the extruder were quenched in liquid nitrogen. The frozen sample (ca. 15g) was grinded in a mill (Janke & Kunkel IKA-A10; F.R.G.) resulting in a powder. The by-products and reactants were extracted with 100 cm^3 methanol in which 10 cm^3 titrisol buffer (pH 7) was dissolved. The resulting mixture was neutralised with hydrochloric acid. The precipitated starch was filtered over a *Büchner* funnel with a Schleicher & Schüll Filter N° 589 and washed with 50cm^3 methanol and then vacuum dried (12h, 60°C).

2.3 Measuring the *MS* of acetylated starch by titration

Deacetylation of acetylated starch will go to completion in diluted aqueous sodium hydroxide solutions. This property can be used to measure the amount of acetyl groups in acetylated starch. About 0.7 g dry acetylated starch was weighted accurately and added to 25 cm^3 demineralised water. The pH of the obtained solution was measured and 25 cm^3 0.1 N sodium hydroxide solution was added. After 12h the solution was titrated back with 0.1 N HCl down to its original pH prior to the NaOH addition. The *MS* followed from an iterative procedure:

$$M_{AGU} = \frac{m_{dryed\ acetylated\ starch}}{(Mw_{AGU} + MS\ Mw_{Acetyl})} \quad (1)$$

$$MS = \frac{M_{Acetyl\ groups}}{M_{AGU}} = \frac{(m/Mw)_{NaOH,\ added} - (m/Mw)_{HCl,\ added}}{m_{starch}/Mw_{AGU}} \quad (2)$$

With $M_{W,\ Acetyl}$, the molecular mass of the acetylated group, M_{AGU}, the amount of starch moles present in the dryed acetylated starch, (m/M) is the number of moles of a component, $M_{w,\ AGU}$ is the molecular mass of one anhydroglucose unit and m_{starch}, the mass of anhydroglucose units in the final solution. Iterative solving of eqns. 1-2 stopped when $|MS_{new}| \leq 0.001\,|MS_{old}|$.

2.4 Measuring of the *MS* of hydroxypropyl starch by the *Johnson* method

The *MS* of hydroxypropylated starch was determined using the *Johnson* method [8]. The hydroxypropyl group of starch was hydrolysed to propylene glycol which in turn was dehydrated to propionaldehyde and the enolic form of allyl alcohol. The products were reacted with ninhydrin (1,2,3-triketo-

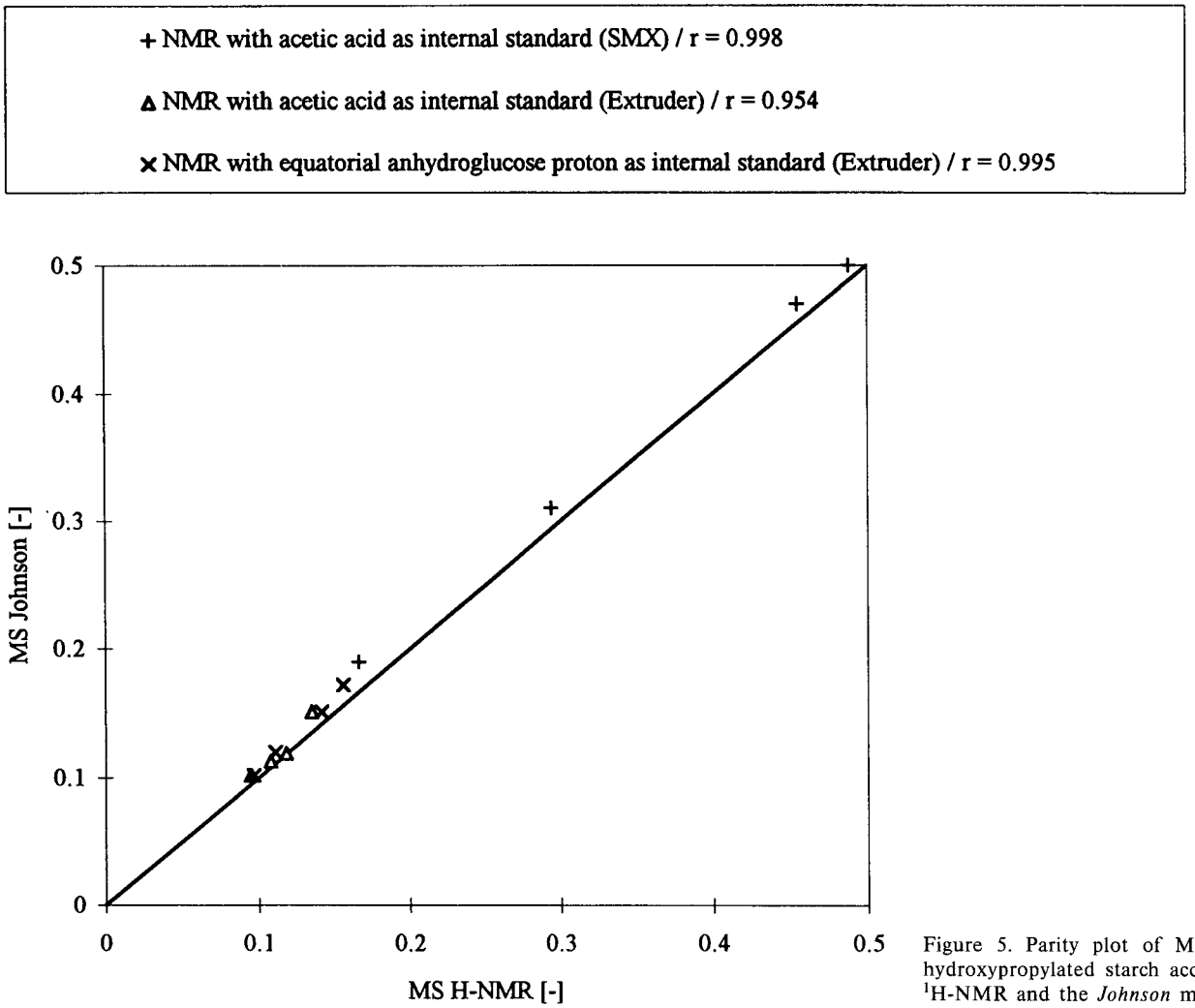


Figure 5. Parity plot of MS analysis of hydroxypropylated starch according to the ^{1}H-NMR and the *Johnson* method.

hydrindene monohydrate) forming a purple coloured complex. The amount of propylene glycol could be determined spectrophotometrically.

2.5 Measuring of the *MS* of acetylated or hydroxypropylated starch by ^{1}H-NMR

Depending on expected *MS* 0.01 to 0.05 g. of dry modified starch (acetylated as well as hydroxypropylated) was dissolved in 2 cm^3 D_2O. Two analysing techniques were used to obtain the *MS*. The first technique uses acetic acid and t-butanol as an internal standard, for hydroxypropylated starch and acetylated starch, respectively. To these samples the appropriate internal standard was added until 10 mg internal standard/g D_2O was reached. The amount of internal standard was adjusted in such a way that the NMR peak ratio of the signals of the internal standard and the acetyl or hydroxypropyl group was equal to one. Alternatively, the equatorial proton of starch (Fig. 2) was used as an internal standard. This peak can be found at 5.4ppm and depends linearly on the amount of anhydroglucose units present in the sample. Then 0.01 g of dry modified starch was dissolved in 2 cm^3 D_2O. Vigorous shaking resulted in a clear solution, which was transferred to an NMR-tube. Good spectra were obtained from 32 pulses with a delay of 5s between each pulse.

3 Results and Discussion

Figures 2, 3 and 4 show spectra for native gelatinised, acetylated and hydroxypropylated starch, respectively. For the chemically modified starches, the respective internal standards were added. Acetic acid and tert-butanol have characteristic proton signals at 2.0ppm and 1.2ppm, respectively. Hydroxypropyl starch and acetylated starch have characteristic proton signals at 1.2ppm (a doublet) and 2.1ppm, respectively. The surface area of the characteristic proton of the substituent group and of the internal standard was calculated by numerical integration. The moles of tested hydroxypropyl starch was determined from:

$$M_{HPS} = \frac{m_{starch}}{(Mw_{AGU} + (MS_{assumed} \cdot Mw_{PO}))} \quad (3)$$

with m_{starch} : amount of hydroxypropyl starch in test sample,
$M_{w,\,AGU}$: molecular weight of one anhydroglucose unit,
$MS_{assumed}$: assumed MS,
$M_{w,\,PO}$: molecular weight of the attached hydroxypropyl group,
M_{HPS} : amount of moles of hydroxypropyl starch.

A new *MS* was calculated from:

$$MS_{new} = \left(\frac{M_{HAc}}{M_{HPS}} \frac{I_{PO,\,HPS}}{I_{HAc}}\right) \quad (4)$$

with M_{HAc} : amount of moles of acetic acid in the sample,
$I_{PO,\,HPS}$: integrated signal of the NMR peak from the hydroxypropyl group (Figure 3),

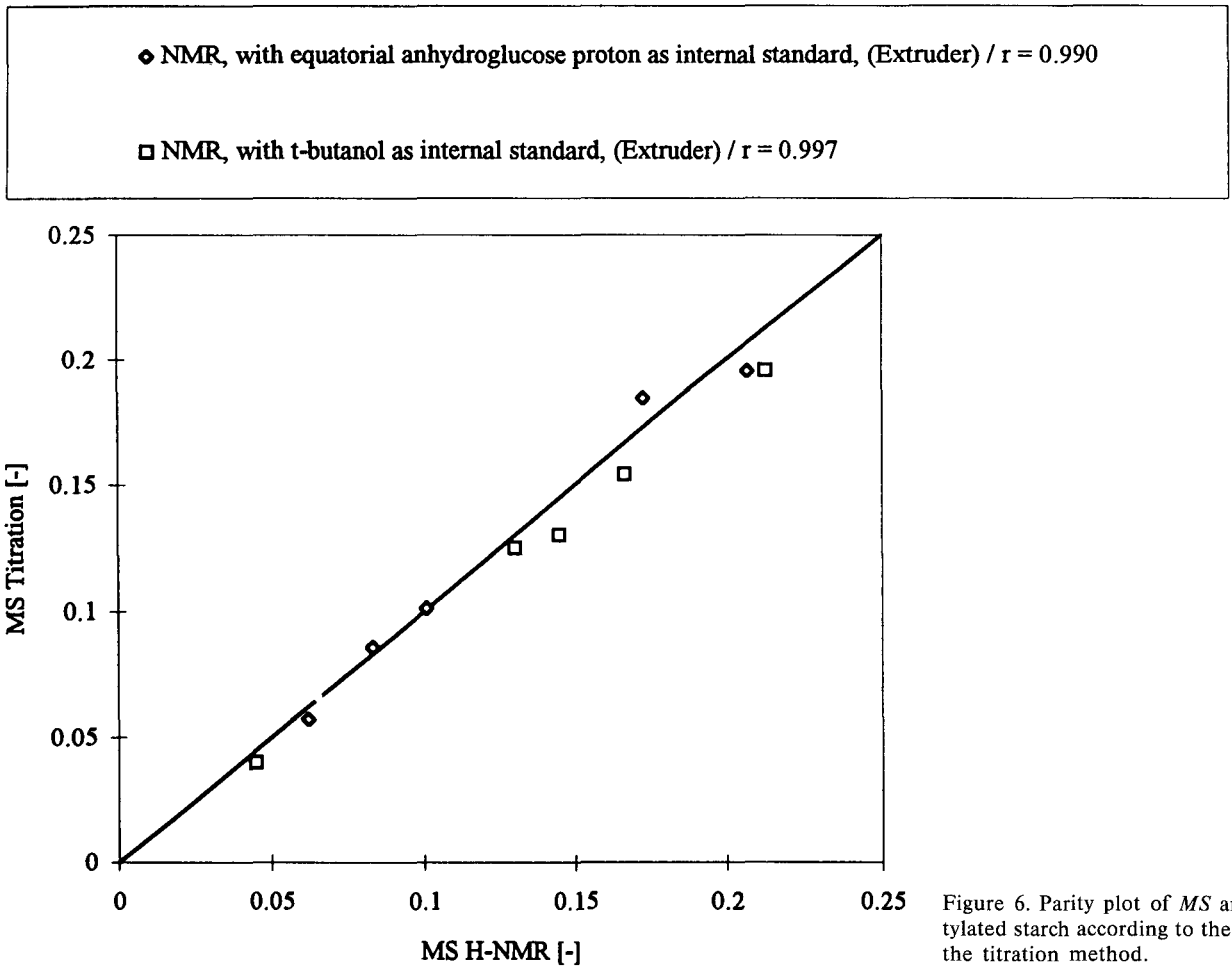


Figure 6. Parity plot of *MS* analysis of acetylated starch according to the ^{1}H-NMR and the titration method.

Table 1. *MS* Values Obtained from H-NMR and from *Johnson*'s Method.
a) Samples produced by the static mixer. b) and c) Samples produced by the extrusion process.

Hydroxypropylated starch		
Static Mixer		
a)	*MS* (NMR with acetic acid) (as internal standard) (–)	*MS* (*Johnson*) (–)
	0.166	0.19
	0.455	0.47
	0.294	0.31
	0.488	0.5
Co rotating twin screw extruder		
b)	*MS* (NMR with acetic acid) (as internal standard) (–)	*MS* (*Johnson*) (–)
	0.146	0.172
	0.135	0.151
	0.105	0.12
	0.118	0.113
	0.108	0.119
	0.095	0.102
c)	*MS* (NMR with equatorial AGU proton) (as internal standard) (–)	*MS* (*Johnson*) (–)
	0.156	0.172
	0.142	0.151
	0.111	0.12
	0.097	0.102

I_{HAc} : integrated signal of the NMR peak from the acetic acid group,
MS_{new} : newly assumed *MS*.

This procedure continued until $|MS_{assumed} - MS_{new}| \leq 0.001$. Alternatively, the equatorial proton of starch (Fig. 1) was used as an internal standard. This peak can be found at 5.4ppm and depends linearly on the amount of anhydroglucose units present in the sample. The *MS* can be calculated directly from:

$$MS = \frac{I_A}{3I_{AGU}} \quad (5)$$

In which I_A : area of the NMR peak from the hydroxypropyl, or the acetyl group of the starch,
I_{AGU} : area of the NMR peak from the equatorial proton of the anhydroglucose unit of starch. This peak area is multiplyed by 3 due to the fact that three reactive sites are present at one anhydroglucose unit.

Experimental results from the different analysis methods are presented in Tables 1 and 2. Figures 5 and 6 present the parity plots of experimentally obtained *MS* of the hydroxypropylated- and the acetylated-starch, respectively. For HPS, the ^{1}H-NMR method and the *Johnson* method were in good agreement. Deviations between the two methods can be explained from the sensitivity of the *Johnson* method to experimental errors. From proton NMR, the absolute error was $\pm 0.01 MS$.

Table 2. *MS* Values Obtained from H-NMR and from Titration. In both cases a and b the samples were produced by an extruder.

Acetylated starch		
Counter rotating twin screw extruder		
a)	*MS* (NMR with equatorial AGU proton) (as internal standard) (–)	*MS* (Titration) (–)
	0.109	0.116
	0.101	0.101
	0.083	0.086
	0.062	0.057
	0.048	0.057
	0.128	0.114
	0.173	0.185
	0.207	0.196
b)	*MS* (NMR with t-butanol) (as internal standard) (–)	*MS* (Titration) (–)
	0.045	0.040
	0.130	0.125
	0.145	0.130
	0.167	0.154
	0.213	0.196

Comparing the *MS* (Fig. 5) of acetylated starch measured by proton NMR and by titration, shows a slight deviation. This difference can be attributed to the determination of the exact end point in the titration technique. Because starch acts as a weak polyacid [9, 10] it buffers the sample solution. Determining the exact end point becomes more difficult this way. The results of proton NMR using acetic acid or t-butanol as internal standard do not differ significantly from the results obtained from proton NMR using the equatorial proton of the starch anhydroglucose unit as an internal standard.

4 Conclusions

^{1}H-NMR spectroscopy can be used as an easy and accurate tool for analysis of the *MS* of acetylated and hydroxypropylated starch. For hydroxypropyl starch, *MS* results were in good agreement with results obtained from the *Johnson* method. This technique was tested for $0.04 < MS < 0.5$. For acetylated starch, the technique was tested for $0.03 < MS < 0.25$. Titration was used as a reference. The equatorial proton of the anhydroglucose unit of the starch can be used as an internal standard for the *MS* analysis of hydroxypropylated as well as acetylated starch. Results from this method are comparable to analyses with acetic acid or t-butanol added as an internal standard.

Nomenclature

MS	: Molecular Substitution	(–)
M	: concentration	(mole/m^3)
I	: area under the NMR peak	(–)
m	: mass	(g)
M_W	: molecular mass	(mole/g)

Acknowledgement

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eration and analysis of part of the samples with the *Johnson* method. Dr. *R. Huls* and Mr. *W. Beukema* are acknowledged for there advice in interpreting NMR-spectra and trusting us using the NMR equipment.

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Address of authors: Ir. *R. A. de Graaf,* Dr. *G. Lammers,* Prof. Dr. *L. P. B. M. Janssen,* and Prof. Dr. *A. A. C. M. Beenackers.* University of Groningen, Nijenborgh 4, 9747 AG, Groningen (The Netherlands). Pheon 050-3634485 or 050-3634484. Fax 050-634479.

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Abbaubare Polymerwerkstoffe auf der Basis nachwachsender Rohstoffe – Möglichkeiten und Grenzen

H.G. Fritz, W. Aichholzer, T. Seidenstücker und B. Widmann, Stuttgart (Deutschland)

Der Beitrag beschreibt Rezeptierungs- und Aufbereitungsmöglichkeiten für die Herstellung bioabbaubarer Polymerwerkstoffe unter Einbeziehung nachwachsender Rohstoffe. Diskutiert werden die Inkorporierung nativer Stäkre in aliphatische Polyesterurethane sowie das Abbauverhalten derartiger gefüllter Polymersysteme. Erörtert werden weiterführend die Generierung Thermoplastischer Stärken (TPS) mittels Zweischneckenkneter und deren Einsatz als Matrixkomponenten für flachsfaserverstärkte Composites. Die Aufbereitung von Polymerblends aus Thermoplastischer Stärke und biochemisch abbaubaren, synthetischen Thermoplasten führt zu interessanten Zweiphasenwerkstoffen, denen ein beträchtliches Entwicklungspotential zugeschrieben werden kann. Augrund enggeknüpfter Struktur/Eigenschaftsbeziehungen kommt der Morphologieausbildung beim Compoundierungsprozeß entscheidende Bedeutung zu. Dimensionslose Kennzahlen zum Abschätzen und Beurteilen der sich einstellenden Phasenstruktur werden vorgestellt.

Biodegradable Polymer Systems Based on Renewable Raw Materials – Chances and Limitations. This contribution describes formulation and preparation concepts for the generation of biodegradable polymer systems under the inclusion of growing-again raw materials. The incorporation of native starch into aliphatic polyurethanes as well as the degradation behaviour of such filled systems will be discussed. In a further chapter, concepts for the generation of thermoplastic starch using a twin-screw kneader, will be presented. These destructurized polysaccharides can form the polymer matrix of flaxfibre reinforced composites. The preparation of polymer blends, composed of thermoplastic starch and biodegradable synthetic thermoplastics leads to competitive two-phase polymers including a remarkable potential of development. As a result of close-meshed structure/property interrelationships, the morphology formation during the compounding process is of utmost importance. Dimensionless characteristics will be presented that can be used to estimate and to assess the resulting phase structures.

1 Einführung

Kontinuierlich wachsende Müllberge, der knapper werdende Deponieraum und an ihren Kapazitätsgrenzen arbeitende Müllverbrennungsanlagen sind Antrieb auf der Suche nach effizienten und ökologisch vertretbaren Müllentsorgungskonzepten. Da synthetische Kunststoffe sich in großen Volumenanteilen im Hausmüll wiederfinden, wird dieser Stoffgruppe kritische Aufmerksamkeit zuteil. Aus dieser prekären Situation heraus wurde wiederholt die Verwendung abbaubarer Kunststoffe zur politischen Forderung erhoben.
Begründet wird diese zum einen mit den Argumenten einer Erdölressourcenschonung und CO_2-Emissionsreduktion, zum anderen steht im Hintergrund die Hoffnung, daß man, zumindest partiell, das Entsorgungsproblem den natürlichen Degradationsprozessen überlassen könnte. Schließlich würden sich aus der Tatsache, daß die Grundkomponenten vieler biochemisch abbaubaren Kunststoffe nachwachsenden Rohstoffen entstammen, neue Absatzmärkte für die Agrarwirtschaft ergeben.
Im folgenden soll anhand neuer Werkstoffentwicklungen aufgezeigt werden, wie realistisch derartige Vorstellungen sind [1].

2 Ausgangssituation

1990 wurden in der Bundesrepublik Deutschland (alte Bundesländer) 10,2 Mio Mp Polymerwerkstoffe produziert (Abbil-